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Autor: Fölsch, Detlef W. / Liau, Ming-Yi

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Department of Veterinary Medicine (Prof. and Head: Y. P. Liu),
College of Agriculture, National Taiwan University (N.T.U.)

Experimental Infection, Clinical-Hematological Course, and Isolation of *Anaplasma marginale* and *Paranaplasma* of Taiwan Origin

DETLEF W. FÖLSCH * and MING-YI LIAU **

Abstract

A total of 6 experimental cases of anaplasmosis using *Anaplasma marginale, Paranaplasma discoides* and *P. caudata* of Taiwan origin were studied in splenectomized calves. The environment and condition of 2 calves (No. 51, 57) during the experiment were those normally found in rural Taiwan. Anaplasmosis initiated a severe anemia in all animals. The result of a single CA-test (ANATEST) was unreliable as a diagnosis of anaplasmosis. Naturally occuring *Anaplasma* infection in deer was not shown.

Introduction

The main difficulties in rearing and keeping cattle for production of milk and meat in Taiwan are lack of experience in management, and the various diseases in ruminants. Import of cattle and the following time of adaption brings losses as recently published by OTTE (1968).

Intensive work in the veterinary field has been done in previous years as seen in the titles which are summarized by YAMANE in the Japanese bibliography of Animal Husbandry and Veterinary Medicine in Taiwan, 1904–1945. Hemoprotozoan parasites *(Babesia, Theileria, Piroplasma* and *Trypanosoma)* and their occurrence in Taiwan have been described in detail.

OGURA (1969) stated that blue stained particles similar to *Anaplasma* in size, colour and situation in the erythrocyte were recognized in past years; and he cited THEILER, who underlined, too, that the morphology of *Theileria mutans* in the first 20 days of infection allowed no distinct differentiation from *Anaplasma.* At that time no serological test was elaborated for further proof of anaplasmosis.

The purpose of the following investigation was to isolate the most severe and pathogenic anaplasma-like organism of Taiwan origin and to observe its clinical and hematological course in calves kept under control.

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* Visiting postdoctoral fellow of the German Academic Exchange Service (DAAD) and the Ministry of Education of the Republic of China. Present address: 24 Lübeck, Goethe-Strasse 14, Germany.

** The work was carried out while M. Y. Liau was in his senior year of the Department of Veterinary Medicine, National Taiwan University.

Materials and Methods

Calves. The calves (Holstein) used in this experiment were obtained from the farm of the Animal Husbandry Department of N.T.U. (National Taiwan University). They were moved to the stable of the Veterinary Hospital of N.T.U. Calves numbered 66,65 (non-identical twins), 299 and 298 were kept in a stable protected against flies and mosquitos by mosquito gauze and spraying with insecticides. No. 51 and No. 57 were kept in another box-stall without screen.

Management. The calves were fed optimally with green grass and concentrate.

Since the balance in the Veterinary Hospital was out of order, only the body weight (b.w.) on the day of buying and that of the carcass could be measured exactly by scales. Otherwise a tapemeasure was used to get an estimation of increase or decrease in animals' weight.

Control. During the whole time of hospitalization, all animals were checked daily. Temperature, pulse and respiration as well as obvious alterations of the normal condition were registered. Urine was collected and controlled especially during the infectious phase.

Sampling and hematological examination. On two fixed days a week blood was drawn from the jugular vein and blood smears were prepared during the bleeding.

Of each smear 500 erythrocytes (RBC) were counted and the number of infected RBC was expressed as percent. Infected RBC were grouped according to the central or marginal situation of the anaplasma-like bodies. Those bodies touching the margin of the RBC were decided to be *Anaplasma marginale* and all the others *A. centrale,* according to SCHINDLER et al. (1966).

Red blood cell (RBC) and white blood cell (WBC) counts were evaluated by means of an improved Neubauer Levy-chamber; blood cell differentiation was done on Giemsa stained smears. The hemoglobin (Hb) was determined by the method of Sahli; the icterus index (II) estimated by matching the plasma colour in the hematocrit with standards from potassium dichromate. The packed cell volume (PCV) was measured by centrifugation in Wintrobe's tube at 3,000 r.p.m. for 30 minutes.

Capillary tube agglutination test (CA-test) and *Staining techniques* have been described previously in greater detail (FÖLSCH, 1970). Routine staining was done with Giemsa's solution overnight (10–12 h). For observation and differentiation of the forms of anaplasma-like bodies (a.l.b.) Schalm's new methylene blue technique was used (SCHALM, 1964).

Splenectomy. Calf No. 66, 65 and 299 were splenectomized [1] according to the technique of RAYNOUD (1961).

Experimental procedures

Calf No. 66 (Fig. 1), male, 6 months old and with a body weight (b.w.) of 120 kg was in excellent condition. When hospitalized (Nov. 22nd) it showed a normal blood status; no a.l.b. (FÖLSCH, 1970) were visible and the CA-test was negative. Splenectomy was performed 1 week after hospitalization. A day before inoculation of blood infected with anaplasma 6.0 cc of Berenil [2] were injected to prevent the possible transmission of other blood parasites especially *Piroplasma bigemina.* A second Berenil injection was done 10 days later.

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[1] The splenectomy was performed by Prof. C. H. Chang, Large animal clinic, Vet.-Dept., N.T.U.

[2] Berenil, Farbwerke Hoechst AG, Frankfurt, Germany.

Fig. 1. Clinical-hematological data during the course of parasitaemia in the artificially infected calf No. 66.

Date	November 1968		December 1968						January 1969			
	22	28	11	12	14	17	21	24	4	14	16	17
Erythrocytes (million)	6.75				8.48	9.0		8.87	5.25	3.55	1.5	1.21
Erythrocytes' morphology									Anisocytosis Polychromasia	Erythrophagocytosis	Blood looks watery	
Hemoglobin (g/dl)	11				11	11		11.5	7.5	5.0	2	2
Packed cell volume (%)	33				41	42		46	28.5	18	7.5	7
Leukocytes (thousand)	9.4				11.4	8.3		7.2	12.1	18.1	24.3	16.2
Physical condition	Twin, male 6 months 120 kg b.w.		Discharge from both eyes for 3 weeks							Lying down, ears hanging, decreased appetite, mucous membrane pale		107.5 kg b.w.
Temperature (°C) (early morning)		38.8			38.5	37.0		38	38	39.3	40.9	38
Inoculation		Splenectomy	Infectious blood totally 136 cc i.v./s.c.									Death during administration of glucose i.v.
Treatment			Berenil 6.0 cc				Berenil 7.5 cc				Tetracycl.	
Bloodparasites (%) anaplasma-like bodies								Neg.	3.8	70	71	37
E. wenyoni									64			
CA-test								Neg.	Neg.	+	+	

Fig. 2. Clinical-hematological data of calf No. 65 during a natural infection and later during a challenge infection.

Date	January 1969				February 1969					March 1969		April 1969		
	10	24	27	30	1	4	7	11	28	13	28	1	4	15
Erythrocytes (million)	5.75	8.7	5.29	2.62	2.36	3.43	3.6	5	7		5.24	4.62	4.85	7.28
Erythrocytes' morphology					Anisocytosis, polychromasia, basoph. aggreg.						Erythro-phago-cytosis	Anisocytosis, monochromasia		
Hemoglobin (g/dl)	10.5	8.5	5	3	5	5.5	7.5	8	9.5		6.2	5	6.5	7.5
Packed cell volume (%)	35.5	31	24	9.5	17	18	23	27	42		21	18.5	24	21
Leukocytes (thousand)	5.7	6.2	6.28	10.1	10.9	10.3	8.5	9.2	10.2		12.9	13.7	10.2	11
Physical condition	Twin, male 6 months 125 kg b.w.		Decreased appetite, lying down, constipation, muc. membranes pale		Good appetite – 162 kg b.w.									193 kg b.w.
Temperature (°C) (early morning)	38	40.2	40.4	40.5	38	39	38.8		38.5		39.5	38.5	38.5	38.5
Inoculation										Pooled blood 100 cc s.c.				
Treatment	Splen-ectomy			Tetra-cycline 1.5 mg Predni-solon 125 mg										
Bloodparasites (%) anaplasma-like bodies	5	31	42	35	16	3.4	2.4	1.6	3.6		12.8	6	3.6	3.2
E. wenyoni		45	70	65	23	10.2	36	90	4.4			2.6	5.8	0.8
Th. mutans											19.2	6	8	0.8
CA-test	++	Neg.	Neg.	Microfine agglutination		+	+	+	Neg.		+(+)	++	++	+

Fig. 3. Clinical-hematological changes in the artificially infected calf No. 298.

Date	January 1969				February 1969				March 1969				April 1969
	16	24	27	30	1	4	11	28	7	12	14	25	15
Erythrocytes (million)	8.51	6.91	6.45	3.19	1.79	3.93	4.83	6.74	6.29	5.58	4.96	7.07	7.72
Erythrocytes' morphology		Erythro-phago-cytosis		Erythro-phago-cytosis	Anisocytosis, basophilic aggreg., hypo-chromasia								
Hemoglobin (g/dl)	10.5	10.5	4.5	3.3	4	5	8.5	7	8.5	7	5.5	8	8.2
Packed cell volume (%)	34	26	20	14	17.5	23	30	32	39	32	25.5	31	41.5
Leukocytes (thousand)	8.9	8.7	6.8	6.9	8.7	8.9	7.4	11.5	12.75	12.7	11.4	11.4	11.0
Physical condition	Female 7 months 120 kg b.w.	Decreased appetite											181 kg b.w.
Temperature (°C) (early morning)		38.2	39.1	39.4	39.1	38.7		39	38.9	38.1	38.4	38.6	38.1
Inoculation	Blood of calf Nr. 66 with 70% a.l.b.												
Treatment													
Bloodparasites (%) anaplasma-like bodies		20	18	11	1.4	1	5.2	0.6	2.2	6	8.6	0.8	1.6
E. wenyoni		44	90	50	7.4	4	51	0.2	0.2		1.8		0.2
CA-test	Neg.	Fine aggl.	Neg.	Fine aggl.	Neg.	Fine aggl.	+	++(+)	+++	+++	+++	+++	+++

Fig. 4. Hematological findings and the status of chronic anemia of the artificially infected calf No. 51.

Date	January 1969		February 1969				March 1969			April 1969	
	16	27	4	11	21	28	7	21	28	4	15
Erythrocytes (million)	8.76	5.6	2.18	3.9	3.44	2.75	3.75	4.02	4.84	6.47	7.48
Erythrocytes' morphology		Erythro-phago-cytosis			Target cells basophil. aggregations						
Hemoglobin (g/dl)	11.5	6.5	4.5	7.5	5.5	6	7	6.5	8.4	8	8
Packed cell volume (%)	34	21	14	23	17	19	23	23	25	35	35.5
Leukocytes (thousand)		7.2	8.1	7.5	9.1	11.1	7.4	10.7	11.8	12.2	10.9
Physical condition	Female 3 months 90 kg b.w.		Nasal discharge Strong lacrimation of both eyes Nostrils dry								135 kg b.w.
Temperature (°C) (early morning)		38.8	40.4	39.3	39.8	39.0	38.5	38.7	38.9	38.8	38.8
Inoculation	Blood of calf No. 66 with 70% a.l.b.										
Treatment								Tetracycline 1.0 g for 4 days			
Bloodparasites (%) anaplasma-like bodies		26	0.8	7.6	6	5	11.8	6	1.8	2.4	0.6
E. wenyoni		52	8.6	30	4	2	9.4				
CA-test	Neg.	Fine aggl.	Fine aggl.	+	++	++(+)	+++	+(+)	+++	+	Microfine agglutin.

Fig. 5. Clinical-hematological data of the artificially infected calf No. 57.

Date	January 1969			February 1969				March 1969			April 1969
	16	27	30	1	7	11	18	12	21	28	15
Erythrocytes (million)	9.1	6.92	3.83	2.61	2.29	3.25	4.13	6.87	5.3	6.69	8.41
Erythrocytes' morphology							Target cells Leptocytes				
Hemoglobin (g/dl)	10.5	7.5	3.8	4.5	4.9	6.5	6.5	8	6	7	10
Packed cell volume (%)	34.5	17	12	15	16	18	27	37	26	28	41
Leukocytes (thousand)	5.4	7	17.9	8.4	8.8	7	9.6	10.05	12.05	14.8	11.3
Physical condition	Male 3 months 90 kg b.w.	99 kg b.w.	Appetite decreased Lying down Enlarged testes		96 kg b.w.						128 kg b.w.
Temperature (°C) (early morning)		38.8	39.7	39.5	39.5	39.9	39.0	37.9	39.1	38.6	38.7
Inoculation	Blood of calf No. 66 with 70% a.l.b.										
Treatment						Terramycin 0.75 g for 5 days			Tetracycline 1 g for 5 days		
Bloodparasites (%) anaplasma-like bodies		22	27	13	6.8	12.6	4.4	0.4	1.8	9.2	0.8
E. wenyoni		54	30	19	6.4	7.4	76				0.8
CA-test	Neg.	Fine aggl.	+	Micro-fine aggl.	+	+	+	Neg.	+	+(+)	Microfine agglutin.

75 cc of blood from 4 known chronic *Anaplasma* infected cattle and 1 *Anaplasma* carrier (No. 65) from 3 areas of Taiwan were inoculated subcutaneously and intravenously between the second and third week after splenectomy (Dec. 12th to Dec. 20th).

Calf No. 65 (Fig. 2) was a male twin calf to No. 66 with a b.w. of 125 kg. When starting the experiment it had a positive reaction to the CA-test with a consistently low percentage (25 a.l.b./500 RBC = 5%) of possibly infected erythrocytes, although it was not artificially infected. The calf also was treated with Berenil twice in 10 days interval. On Jan. 10th splenectomy was performed. The calf was kept 3 months for clinical control and observation.

Calf No. 298 (Fig. 3), female, 7 months old and 120 kg of b.w., negative to the CA-test was inoculated intravenously and subcutaneously with 150 ml of the blood collected on Jan. 14th from calf No. 66 containing a high number (70%) of erythrocytes infected with *Anaplasma marginale* and *Paranaplasma.*

Calf No. 51 (Fig. 4) female and Calf No. 57 (Fig. 5) male, both 3 months old and of 90 kg b.w. and negative to the CA-test were inoculated intravenously and subcutaneously each with a total amount of 100 ml of blood carrying 70% RBC infected with anaplasma organisms drawn from calf No. 66 on Jan. 14th. Calf No. 57 was bought and hospitalized with testes enlarged three times normal as a result of accidental compression caused during emasculation with castration forceps. At the height of the artificially induced *Anaplasma* infection pus was exuded from the scrotal sack and thereafter the size of the testes became normal. Local treatment followed for 10 days, and 0.75 gm terramycin was injected for 5 days.

Results

Calf No. 66 (Fig. 1). Two weeks after the first inoculation a decrease in RBC and PCV occurred. Polychromasia and anisocytosis of erythrocytes were observed (Dec. 28th) and 60% of the RBC were noted to be infected with *Eperythrozoon wenyoni;* the reddish parasites were also free in the serum (Fig. 6) and persisted for 25 days. *E. wenyoni* had disappeared when 24% of 500 checked RBC were parasitized with *Anaplasma.* At this time the CA-test became positive. In the following days the calf became weak with all symptoms of an acute state of infection: Lying down, ears hanging, mucous membranes pale, dry nostrils and decreased appetite. The RBC count went down to 1.2 million, Hb to 2 gm/dl, PCV to 7% and WBC increased to 18.1 thousand at the day of death. The blood was chocolate coloured and watery. The peak of rectal temperature during the course of infection reached 40.5°C.

400 cc of blood were drawn from this calf on Jan. 14th, when 70% of the RBC were found to be infected with *Anaplasma* organisms (Fig. 7). On that day 2 smears stained with Giemsa's and Wright's solution showed 0.4% of 15,000 WBC containing phagocytized RBC infected with *Anaplasma.*

During infusion of glucose with the incorrect instruments air entered the vein and the weak calf died. The b.w. had lost 12.5 kg during the experimental period.

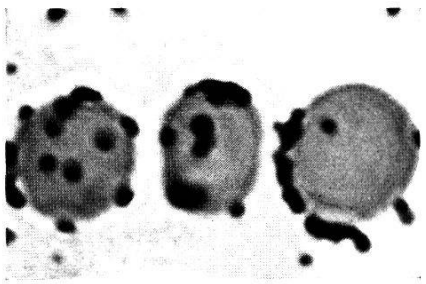

Fig. 6. Calf No. 66, Dec. 28, 1968. 60% of erythrocytes infected with *Eperythrozoon wenyoni*. The parasite is also free in the serum. × 2,000.

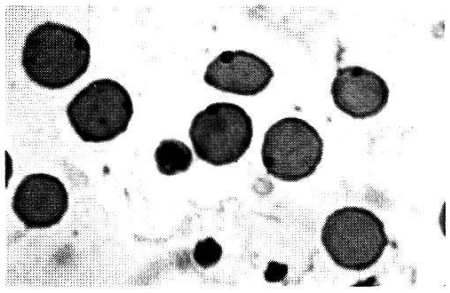

Fig. 7. Calf No. 66, Jan. 14, 1969. 70% of erythrocytes infected with *Anaplasma* organisms. × 1,400.

Calf No. 65 (Fig. 2) exhibited 17 days after splenectomy a decrease of RBC from 5.2 million to 3.4 million, of Hb from 5 to 3 gm/dl and of PCV from 24% to 17% followed with an increased body temperature (40.5°C). 42% RBC carried *Anaplasma* organisms and 70% also *Eperythrozoon wenyoni*. The CA-test then became negative at intervals.

On Jan. 30th 1.5 gm tetracycline and 1.25 mg prednisolon were given in an attempt to prevent loss of the calf.

E. wenyoni was found on Feb. 18th to infect 97% of this animal's RBC's. The parasite count then decreased and its predominance in later determinations alternated with a.l.b.

On March 13th 100 cc of blood sampled from 118 cattle in Kentin- and Nantou-area (FÖLSCH, 1970) were inoculated s.c. for challenge of the unknown antibody titer. The CA-test at that time was negative. 15 days post inoculation the CA-test became positive and 19 days p.i. (Apr. 1st) decrease in RBC to 4.62 million, Hb to 5 mg/dl and PCV to 18.5% were observed as well as erythrophagocytosis.

On March 28th *Theileria mutans*-like organisms (Fig. 8) in ringform were found in a frequency of up to 19.2% in the stained blood smear, and then a continuing improvement took place. Until the end of experiment (Apr. 15/69) a.l.b., *Eperythrozoon wenyoni* and theilerial parasites were visible in the stained smear in a percentage lower than 5, and the CA-test remained positive.

Calf No. 298 (Fig. 3). An incubation period of 8 days was followed by a 20% increase of RBC infected with *Anaplasma* organisms, and a

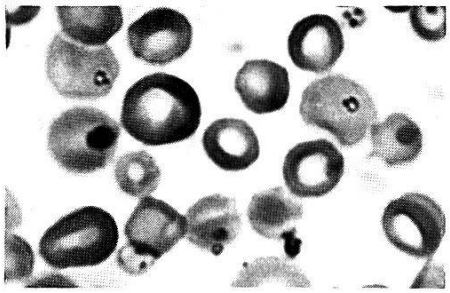

Fig. 8. Calf No. 65, March 28, 1969. 19.2% of the red blood cells infected with *Theileria mutans*-like organisms in ringform. × 1,400.

90% increase in RBC infected with *Eperythrozoon wenyoni*. The latter were also free in the serum. The RBC count dropped to 1.79 million, PCV to 14% and Hb to 3.3 gm/dl. Anisocytosis, basophilic stippling of erythrocytes and hypochromasia as well as a moderate increase in WBC and erythrophagocytosis of *Anaplasma* infected RBC was observed.

11 days after the height of *Anaplasma* infected RBC the CA-test became positive and these reactions became stronger with the course of time. A slight relapse of infection was visible 2 months post inoculation, when the count fell to 4.96 million and Hb to 5.5 gm/dl.

At the end of the 3 months observation time p.i. the calf had gained 61 kg of b.w. The febrile reaction was mild, up to 39.4°C.

Calf No. 51 (Fig. 4). An incubation period of 11 to 14 days was followed by a moderate infection of erythrocytes with *Anaplasma* (26%) and *Eperythrozoon wenyoni* (52%), by a decrease in RBC from 8.76 million to 2.18 million and of Hb from 11.5 to 4 gm/dl. Anisocytosis, basophilic aggregations, target cells and hypochromasia remained for 25 days during the low RBC state. The CA-test became positive 21 days post inoculation.

To depress the chronic state of anaplasmosis 5 weeks after the artificial infection 1 gm tetracycline was injected on each of 3 consecutive days. In spite of this antibiotic treatment 11 weeks were required for the RBC count to return to the normal range. A strong lacrimation of both eyes began 4 weeks p.i. and continued the whole time until the end of observation. Although the appetite was always fairly good the body weight increased in the 3 months from only 90 kg to 128 kg. The maximum rectal temperature was 40.4°C.

Calf No. 57 (Fig. 5). From the 11th day after the artificial infection with *Anaplasma* bodies the existing anaplasmosis was indicated by the following changes in the hematological data: 27% of the RBC were attacked by *Anaplasma* organisms and 54% also with *Eperythrozoon wenyoni*. For 2 months the RBC count remained under the normal rate, and anisocytosis and the presence of target cells were evident.

Treatment with 1.0 gm tetracycline i.m. for 5 days was followed by an increase of blood values to the normal index. The reaction of the CA-test became weakly positive at the beginning of the infection. Microfine agglutination in the whole tube alternated with full positive reactions. Febrile temperature went up to 39.9°C.

Calf No. 299. 6 weeks after the injection of deer blood the observation was ended. The CA-test remained negative and the clinical and hematological values did not change from normal. During this time the animal gained 37 kg of b.w.

Microscopic findings of stained blood smears

The purpose of this part of the study was to make a distinction in the either marginal or central situation of the *Anaplasma* bodies (SCHINDLER et al., 1966). 900 affected RBC of each experimental animal were differentiated. On 3 different days during the period of highest percentage infection (5–9 days), smears stained by two different techniques (Giemsa, Wright) were checked. The results (Fig. 9) of No. 66,

Animal No.	Anaplasma bodies situated	
	marginal	central
66	81	19
298	79	21
51	81	19
66, 298, 51, 57 (artificially infected)	79%	21%
65 (naturally infected)	73%	27%

Fig. 9. Comparative situation of *Anaplasma* bodies in artificially (No. 66, 298, 51, 57) and naturally (No. 65) infected animals.

298, 51, 57 show 79% of the organisms situated marginally (touching the cell margin), and 21% situated centrally. These values are comparable to those from the naturally infected calf No. 65, which had 73% marginal and 27% central *Anaplasma bodies*. SCHINDLER et al. (1966) found 60.4% marginal bodies during experimental infection with a South African strain of *Anaplasma marginale*.

A microscope with calibrated ocular was used to measure the diameter of the *Anaplasma* organisms on Wright- and Giemsa-stained

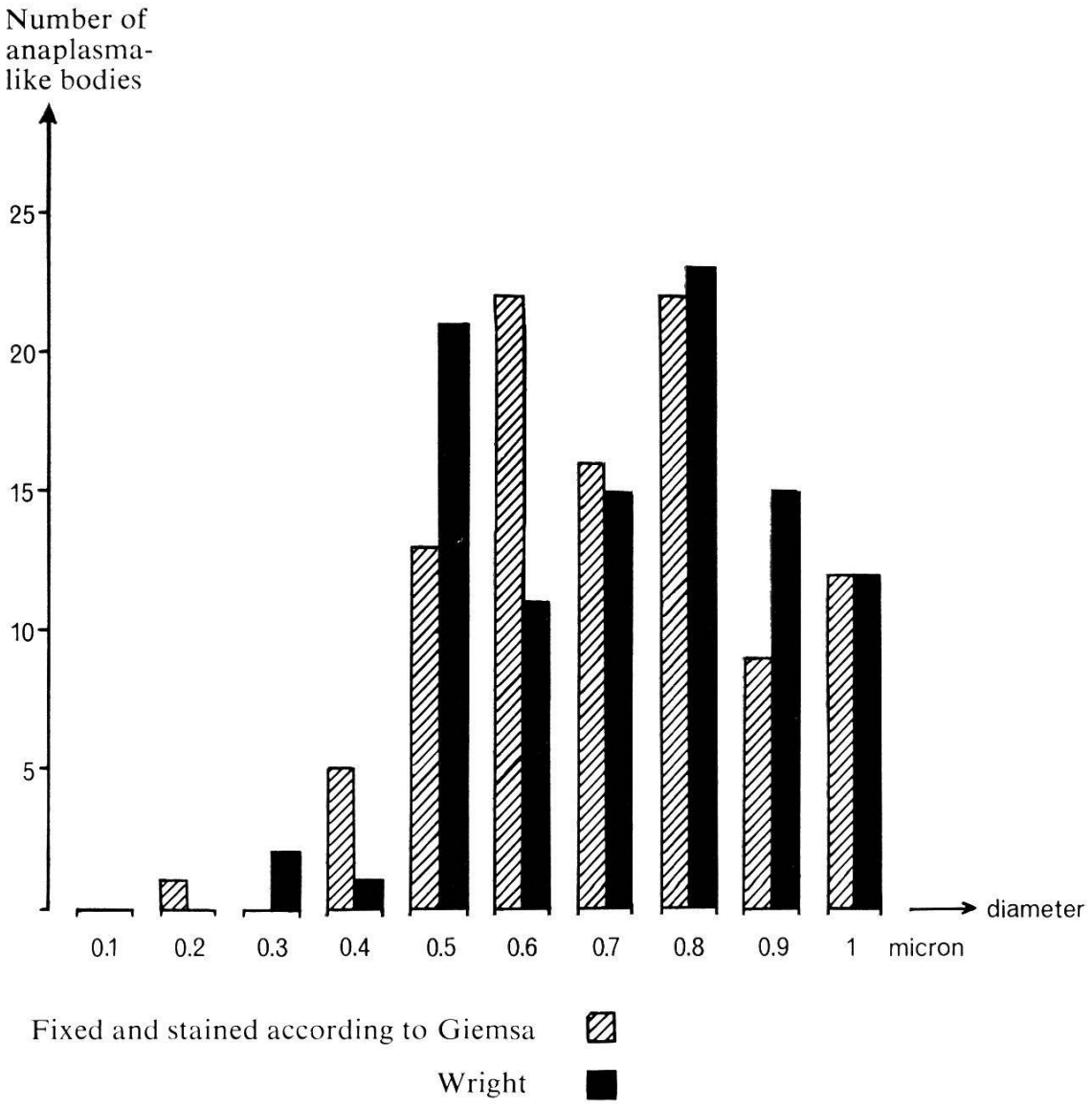

Fig. 10. Diameter of *Anaplasma* bodies. The diameter of the 200 measured bodies lies between 0.7 and 0.8 micron as determined by the moving average method.

blood smears from calf No. 66 when 24% of the RBC showed infection. The diameter of 200 a.l.b. had an average (moving average method) of 0.7 to 0.8 micron (Fig. 10).

Giemsa stained smears from calf No. 66 showed 70% of RBC with a.l.b., dark blue, round organisms. When fresh blood of the same origin was prepared by the new methylene blue vital stain, a technique described by SCHALM (1964), variations in form were visible.

Besides the round *Anaplasma* bodies of approximately 0.8 micron in diameter moving or jumping in the RBC, there were *Anaplasma* organisms with a long tail crossing in total the erythrocyte and appearing either as a drum-stick or dumb-bell (Fig. 11). Others resembled a loop with one or two round bodies at their outskirts (Fig. 12).

All these forms have been found in America by means of fluorescin-labelled antibody studies and cross immunity studies as described by

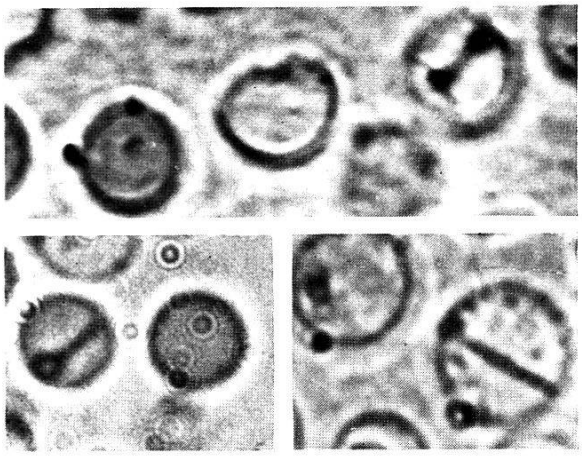

Fig. 11. Calf No. 66, Jan. 14, 1969. *Paranaplasma caudata: Anaplasma* organisms with a long tail crossing in total the red blood cell and appearing as a drumstick and as a dumb-bell. New methylene blue vital stain. × 2,000.

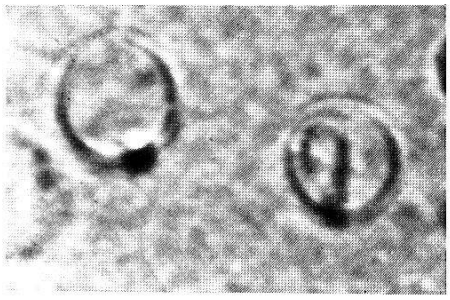

Fig. 12. Calf No. 66, Jan. 14, 1969. *Paranaplasma discoides: Anaplasma* organisms resembling a loop with one or two round bodies at their outskirts. New methylene blue vital stain. × 2,000.

KREIER & RISTIC (1963 a, b, c), who classified them as *Paranaplasma caudata* and *Paranaplasma discoides* in contrast to the round *Anaplasma marginale* organisms without appendages.

Isolation and antigen production

210 cc of blood collected from calf No. 66, which had 71% infected RBC (Jan. 15th), were prepared for CF antigen. The technique of the Servall Distilled Water Extract (Servall antigen) was used according to the outlines of GATES et al. (1954). The procedure was done in the laboratory of W. F. CHEN, D.V.M., U.S. Naval Medical Research Unit No. II, Taipei.

Discussion and Conclusion

During the experimental *Anaplasma* infection the relations between *Anaplasma* organisms visible in the blood, hematological data and clinical behaviour of the animals could be closely followed. The clinical

symptoms alone are too unspecific to diagnose anaplasmosis. Hematurea was never observed.

When expressing the percentage of RBC carrying *Anaplasma* bodies, it must be kept in mind that under natural conditions there are also particles of similar form, size and staining characteristics, such as: *Eperythrozoon* and theilerial parasites, Howell-Jolly-bodies, nuclei of thrombocytes superimposing on erythrocytes, precipitation of serum proteins especially in acutely ill animals, and particles caused by improper staining procedure. A diagnosis of anaplasmosis by microscope therefore is evident only when about 10% or more RBC contain anaplasma-like bodies. Anemia with reduced RBC count and low PCV, and a proof of a.l.b. in a stained blood smear are methods which can be carried out by every veterinarian with ordinary laboratory equipment.

Although the CA-test is a widely used technique, one should be skeptical when decisions are based on a test made only once with a single serum sample from a single animal. Microfine agglutination in the whole tube or complete negative reactions may be observed even in the presence of an *Anaplasma* infection (No. 57).

The challenge dose of *Anaplasma* infected blood administered to the previously, naturally infected calf No. 65 caused a drop of 2 million in the RBC count, even though the animal had recovered 2 months earlier and possessed an antibody titer against *Anaplasma*. This result is probably due to splenectomy of the animal, as its blood (March 28th) was inoculated (March 29th) in a quantity of 80 cc i.v. and 20 cc s.c. in each of the experimental calves No. 51, 57, 298, and did not cause a decrease in the animals' RBC count during an observation time of 18 days.

The calves No. 298, 51 and 57 which were not splenectomized had a chronic anemia for 2 to 2½ months caused by the infection with *Anaplasma marginale, Paranaplasma discoides* and *Paranaplasma caudata*. This chronic depression in the hemapoetic system must be considered as a retarding effect in the growth of calves.

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References

FÖLSCH, D. W. (1970). Survey on *Anaplasma* and anaplasma-like bodies in small and large ruminants in three areas of Taiwan. – J. Taiwan Ass. Animal Husbandry No. 17 (December).

GATES, D. W., MOHLER, W. M., MOTT, L. O., POELMA, L. J., PRICE, K. E. & MITCHELL, J. (1954). A comparison of antigen production methods and complement-fixation procedures for diagnosing bovine anaplasmosis. – Proc. U.S. Livestock San. Ass., 58th Annual Meeting.

KREIER, J. & RISTIC, M. (1963 a). Anaplasmosis. X. Morphologic characteristics of the parasites present in the blood of calves infected with the Oregon strain of *Anaplasma marginale*. – Amer. J. vet. Res., Vol. *24*, No. 101, 676–687.

KREIER, J. & RISTIC, M. (1963 b). Anaplasmosis. XI. Immunoserologic characteristics of the parasites present in the blood of calves infected with the Oregon strain of *Anaplasma marginale*. – Amer. J. vet. Res., Vol. *24*, No. 101, 688–696.

KREIER, J. & RISTIC, M. (1963 c). Anaplasmosis. XII. The growth and survival in deer and sheep of the parasites present in the blood of calves infected with the Oregon strain of *Anaplasma marginale*. – Amer. J. vet. Res., Vol. *24*, No. 101, 697–702.

OGURA, K. (1969). Babesiosis, theileriosis and piroplasmosis. – Lecture in the Vet.-Dept., College of Agriculture, National Taiwan University.

OTTE, E. (1968). Anaplasmosis in Taiwan. II. Import of exotic cattle and premunition with *Anaplasma centrale*. – Berl. Münch. tierärztl. Wschr. *21*, 428–433.

RAYNAUD, J.-P. (1961). Une méthode de splénectomie des bovins adultes par résection de la 12e côte gauche. – Rev. Elev. Méd. vét. Pays trop. *14*, 321–327.

SCHALM, O. W. (1964). A simple and rapid method for staining blood films with the new methylene blue. – J. Amer. vet. med. Ass. *145*, 1184–1188.

SCHINDLER, R., RISTIC, M. & WOKATSCH, R. (1966). Vergleichende Untersuchungen mit *Anaplasma marginale* und *A. centrale*. – Z. Tropenmed. Parasit. *17*, 337–360.

YAMANE, J. & TAKEGAMI, K. (undated). Bibliography of animal husbandry and veterinary medicine in Taiwan, 1904–1945 (Japanese).

Zusammenfassung

Während der bei Kälbern experimentell durchgeführten Infektion mit *Anaplasma* wurde die Abhängigkeit von *Anaplasma*-Organismen im Blut zu den hämatologischen Werten und dem klinischen Verhalten der Tiere genau verfolgt. Das Krankheitsbild der Anaplasmose ist zu unspezifisch, um diese Erkrankung aus der Symptomatik diagnostizieren zu können. Eine Hämaturie wurde in keinem Fall beobachtet.

Es muß daran gedacht werden, daß die zum Ausdruck gebrachte Prozentzahl der *Anaplasma*-Körperchen tragenden Erythrozyten unter natürlichen Bedingungen auch andere Partikel umfaßt mit ähnlicher Form, Größe und Farbe wie: *Eperythrozoon* und *Theileria*, Howell-Jolly-Körperchen, Kerne von Thrombozyten, die sich auf Erythrozyten gelagert haben, Präzipitation von Serumeiweißen besonders bei akut kranken Tieren und Bestandteile, die durch unsaubere Färbetechnik verursacht sind. Die mikroskopische Diagnose «Anaplasmose» ist nur dann begründet, wenn ungefähr 10% oder mehr der Erythrozyten *Anaplasma*-ähnliche Körperchen tragen. Der Nachweis einer Anämie, die mit erniedrigter Erythrozytenanzahl und niedrigem Hämatokrit einhergeht sowie die zusätzliche Feststellung von *Anaplasma*-ähnlichen Körperchen in einem gefärbten Blutausstrich

beruhen auf Methoden, die von jedem Tierarzt mit herkömmlicher Laboreinrichtung ausgeführt werden können.

Obwohl der CA-Test weithin Anwendung gefunden hat, sollte man die Beurteilung nach einer einmaligen Untersuchung an nur einem Tier mit großer Skepsis bewerten. Mikrofeine Agglutinationen in der gesamten Kapillare oder aber auch völlig negative Reaktionen können trotz vorhandener *Anaplasma*-Infektion beobachtet werden.

Kalb Nr. 65 war auf natürlichem Wege an Anaplasmose erkrankt und hatte Antikörper nachweisbar ausgebildet. Die 2 Monate später erfolgte Injektion einer Belastungsdosis von mit *Anaplasma* infiziertem Blut versusachte eine Verminderung der Erythrozyten um 2 Millionen. Diese Reaktion ist wohl auf die der Belastung vorausgehende Splenektomie zurückzuführen, denn das Blut von Kalb Nr. 65 (28. März), in einer Menge von 80 cc i.v. und 20 cc s.c. in jedes der anderen Kälber Nr. 51, 57, 298 injiziert (29. März), verursachte keine Erythrozytenverminderung während einer Beobachtungszeit von 18 Tagen.

Die nicht entmilzten Kälber Nr. 298, 51 und 57 durchstanden während $2^1/_2$ Monaten eine chronische Anämie, die durch die Infektion mit *Anaplasma marginale, Paranaplasma discoides* und *Paranaplasma caudata* hervorgerufen worden war. Diese chronische Belastung des blutbildenden Systems wird als Verzögerungsfaktor für das Wachstum der Kälber angesehen.

Résumé

A l'aide d'infections expérimentales d'*Anaplasma* chez les veaux, il a été possible d'étudier le rapport existant entre ces parasites et l'image hématologique, de même que le comportement clinique des animaux infectés. Les symptômes cliniques de cette maladie sont trop peu spécifiques pour qu'on puisse la diagnostiquer. Une hématurie n'a jamais été observée.

On ne doit pas négliger que dans la nature, le pourcentage des érythrocytes parasités de corpuscules ressemblant aux anaplasmes peut être faussé par la présence d'autres particules de formes, grandeurs et couleurs identiques: *Eperythrozoon* et *Theileria,* corpuscules de Howell-Jolly, noyaux de thrombocytes qui se sont collés sur les érythrocytes, précipités de protéines du serum (fréquents dans le cas de maladies aiguës) ainsi que des impuretés dues à une mauvaise technique de coloration. Le diagnostic n'est justifié que si 10 % (ou plus) des érythrocytes portent des corpuscules identiques à ceux d'*Anaplasma.* L'observation d'une anémie, accompagnée d'un nombre amoindri d'érythrocytes et d'un Hématocrite bas, de même que la présence de corpuscules comparables à ceux de l'anaplasmose dans un frottis de sang coloré, reposent sur des méthodes qui doivent pouvoir être pratiquées dans le laboratoire de chaque vétérinaire.

Quoique le « CA-Test » ait trouvé une large audience, on devrait réserver un jugement basé sur un seul examen et qui ne concernerait qu'un seul animal. Lors d'une anaplasmose, il est possible d'obtenir des microagglutinations fines dans les capillaires, mais aussi des réactions totalement négatives. Le veau N° 65 était malade d'une anaplasmose naturelle et présentait des anticorps. Deux mois plus tard, l'injection d'une dose de sang infecté d'*Anaplasma* provoqua une baisse des érythrocytes à 2 millions. Cette réaction est vraisemblablement due à la splénectomie effectuée avant l'injection, car le sang de cet animal (28 mars) injecté i.v. (80 cc) et s.c. (20 cc) aux veaux N° 51, 57 et 298 (29 mars) ne provoqua chez ceux-ci aucune baisse des érythrocytes pendant 18 mois.

Les veaux non splénectomisés N° 51, 57 et 298 présentèrent une anémie chronique pendant $2^1/_2$ mois, anémie due à *Anaplasma marginale, Paranaplasma discoides* et *Paranaplasma caudata.* Cet ennui chronique pèse sur le renouvellement du sang et est un frein à la croissance des veaux.